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TITLE: Omega-3 Polyunsaturated Fatty Acid Status, Microglial Activation, Stress Resilience, and Cognitive Performance

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

It is widely reported across mammalian species that deficiency in the dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFA) negatively impacts cognitive performance and mood. A plethora of literature also implicates n-3 PUFA deficiency in disorders such as ADHD, PTSD, major depressive and bipolar disorders, and schizophrenia. Defining potential neuronal mechanisms that link n-3 PUFA levels to cognitive and behavioral deficits has important implications given that the trend of the modern diet has been toward reduced n-3 PUFA intake. Here, we propose human and rodent experiments to evaluate whether the anti-inflammation/pro-resolution effects of n-3 PUFA deficiency contribute to the adverse effects on cognitive performance and affect. In addition, these experiments focus on the expression of dietary n-3 PUFA deficiency in late adolescence/young adulthood—a developmentally critical period during which an individual is vulnerable to mood, psychotic and addictive disorders. We will use a positron emission tomography (PET) imaging strategy in humans as a marker of activated microglia in individuals with low and high plasma n-3 PUFA. In parallel animal studies, we will directly measure microglia activation in an animal model of n-3 PUFA deficiency and determine whether supplementation during early adulthood reverses this effect in correlation with behavior.

15. SUBJECT TERMS

Omega-3 fatty acids, microglia, brain inflammation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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1. INTRODUCTION

Background: Dietary deficiency in omega-3 polyunsaturated fatty acid (n-3 PUFA) is a common feature of the modern diet. Across mammalian species, deficiency in the intake of this essential fatty acid negatively impacts the ability to withstand stress and cognitive performance. Accordingly, recent studies in healthy civilian and military populations indicate a strong relationship between red blood cell (RBC) n-3 PUFA levels and a wide range of brain related problems including impaired cognitive performance, and increased anxiety, impulsivity and suicide. Precise brain mechanisms that underlie the behavioral detriments of n-3 PUFA deficiency and whether they can be reversed by supplementation are largely unknown. The overarching goal of this proposed work is to inform of us about specific brain mechanisms by which dietary n-3 PUFA deficiency and supplementation affects brain and behavior. The mechanistic focus will be on immune responses around neurons in brain regions that are critical for stress reactivity and cognitive performance.

Purpose (Aim2): To determine whether an animal model of n-3 PUFA deficiency is associated with brain microglia activation and whether supplementation during early adulthood reverses this effect in correlation with behavior. In an experimental animal model that mimics current western dietary n-3 PUFA deficiency, we have observed behavioral determents that suggest impaired cognitive performance and anxiety. We hypothesize that this dietary deficiency leads to an immunological insult in the brain and propose to use microglia activation as a method of quantification of this insult. Microglia are the residents of macrophage cells and are the first line of immune defense in the brain. Animals will undergo behavioral characterization before the post-mortem microglial measures. Upon establishing that there is microglia activation in brain regions of interest, we will test whether supplementation during early adulthood reverses this insult in correlation with behavior.

Scope: Establish that brain inflammation is a potential mechanism that underlies behavioral impairments in n-3 PUFA deficient diet, and quantify the impact of supplementation on reversing the inflammatory response and restoring the behavioral impairment. This has the potential to inform the clinical testing of oral and parenteral n-3 PUFA formulations as a treatment for the multitude of conditions where neuroinflammation is a focus, ranging from traumatic brain injury and multiple sclerosis to mood disorders and PTSD.

2. KEYWORDS

Omega-3 fatty acids, microglia, brain inflammation

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major goals of the project (aim 2, animal study)

The major tasks listed in the approved SOW (6/2017) with listed milestones and target dates within the first 12 months are included below:

Major Task 1: ACURO Renewal

Major Task 2: Initiation and maintenance of colonies of first and second generation n-3 deficient animals (Timeline target date 2-30 months

Major Task 3: behavioral testing in deficient animals before and after supplementation, and compared to adequate animals in the same age range. There are four subject groups in this Major Task: (1) animals on adequate diet, (2) animals on deficient diet that remain on that diet, (3) animals on deficient diet that shift to, and remain on, an adequate diet "long-term" beginning after weaning, (4) animals on deficient diet that shift to adequate diet "short-term," one week before behavior testing. Behavioral testing for target date 6-12 months included open field, elevated plus maze, and delayed alternation in two of the proposed 4 groups.

Major task 4: anti-Iba1 immunohistological staining to estimate microglial number and activation. Procedures include perfusion and tissue prep after termination of behavior testing, target date 3-26 months followed by histological assessment and analyses target date 24-36 months.

What was accomplished under these goals?

Major task 1:

Awaiting ACURO renewal.

Major task 2 and 3:

Due to safety measures in response to SARS-Cov2, tasks 2 and 3 (animal breeding and behavioral testing) were placed on temporary hold until safe operations could resume. We anticipate to resume Task two in the next 2 weeks and complete tasks 3 and 4 within the next 4-5 months.

Major task 4:

This task was successfully ongoing before SARS-Cov2 laboratory shut down. The tissue was being successfully processed as reported last year. Fluorescent immunohistochemistry was employed for automated microglia quantification and morphometry analysis. This method allows us to quantify microglia activation.

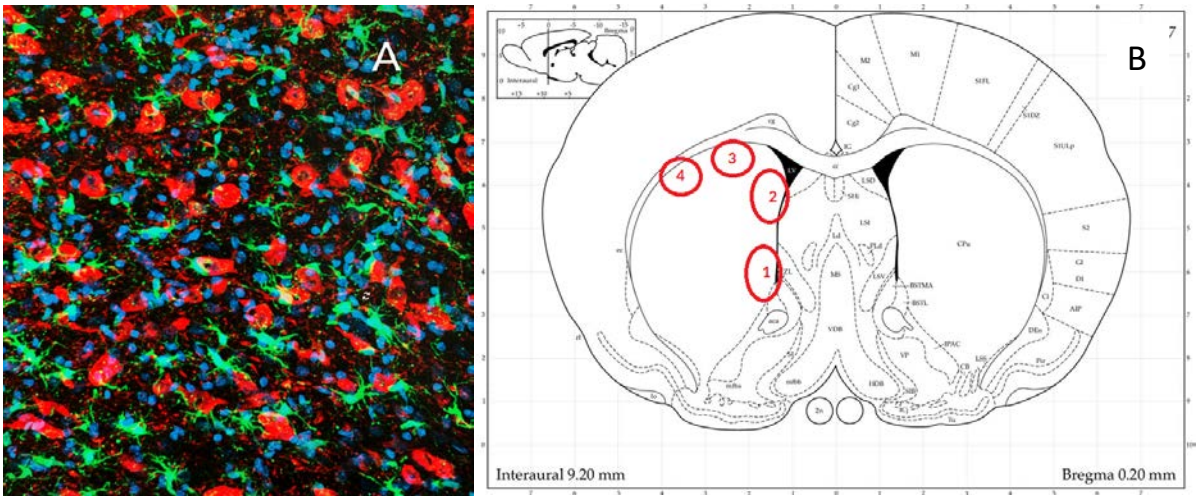

Figure 1. To measure microglial activation in various regions of the Straitum we used a Chicken-anti-TH primary antibody (Abcam, ab76442) combined with Goat-anti-chicken IgY H&L Alexa Fluor 594 (Abcam, ab150176) to visualize TH-containing neurons on the red channel and microglia on the green (Fig 1a). Regions analyzed are circled and numbered (Fig 1b). Cell nuclei are visualized on the blue channel with a DAPI stain (Vector Laboratories, H-1200) (Fig 1a). Staining cell nuclei allows quantification of all cells in an image, as well as determination of the percent of cells in a region that express Iba1 or TH.

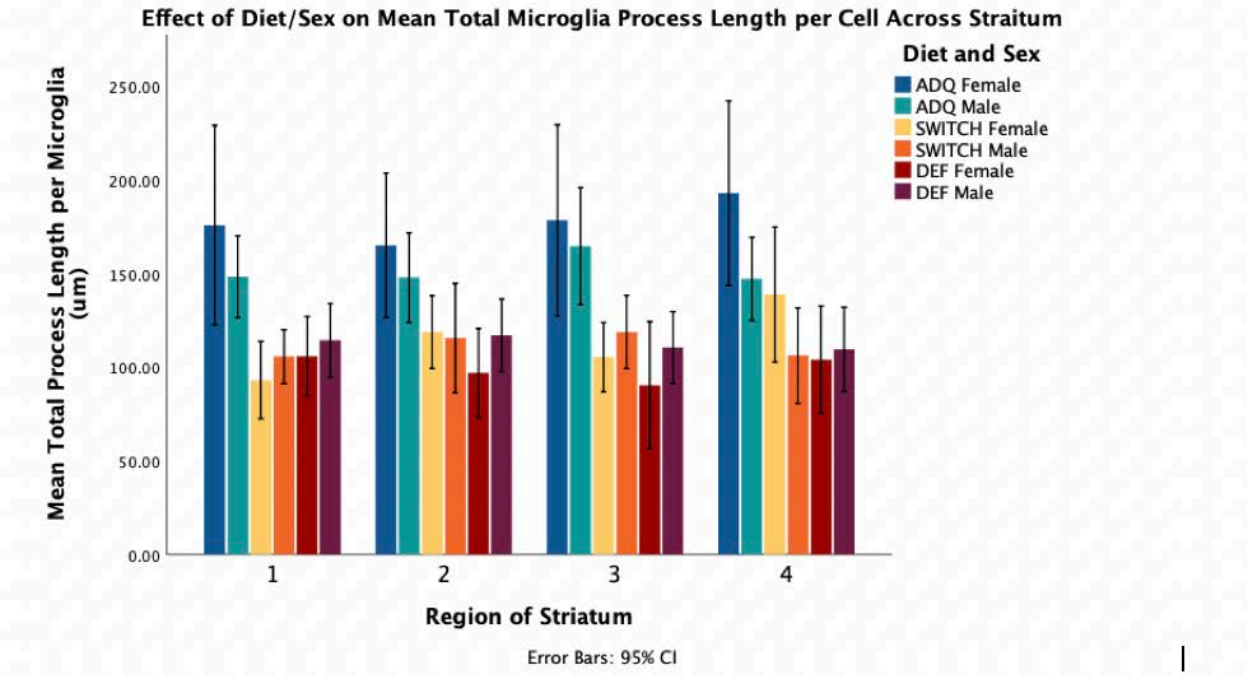

Figure 2. Effect of diet on microglial activation across 4 distinct striatal regions. The y axis depicts the total mean length of microglia processes per cell presenting in both sexes on adequate, deficient, and switched diets. Data was obtained from the four regions of the striatum depicted in Fig 2b. Process length across regions were significantly shorter in deficient diet animals compared to adequate diet p=0.001. No significant differences were noted between striatal regions.

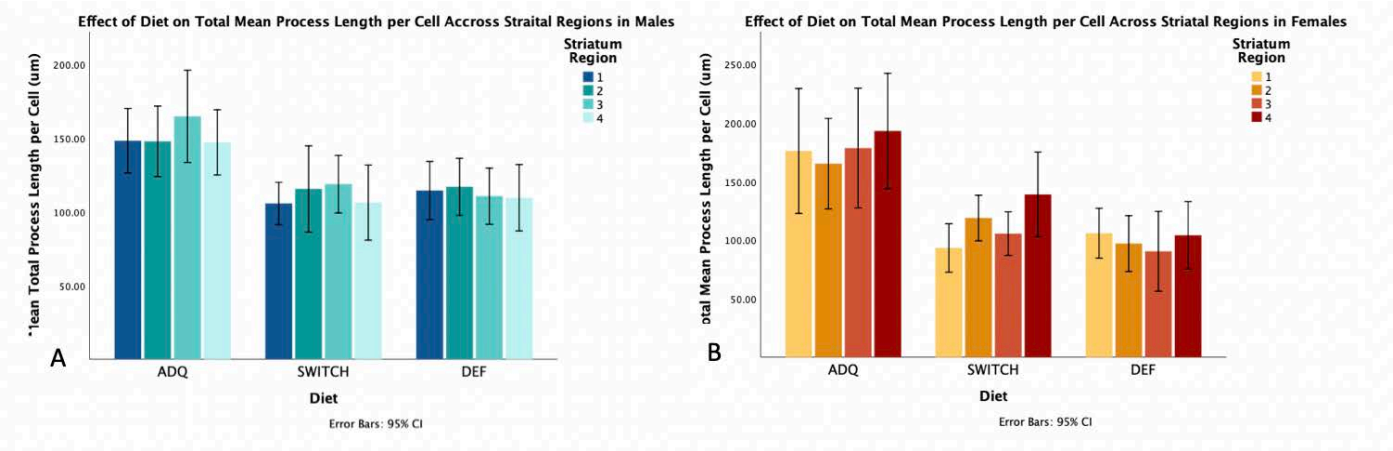

Figure 3. Breakdown of total process length per microglial cell based upon sex. Females exhibited a more pronounced decrease in process length when comparing adequate and deficient diets respectively (p<0.000). No statistically significant differences were observed between supplemented switch diet animals and deficient diet animals in either sex.

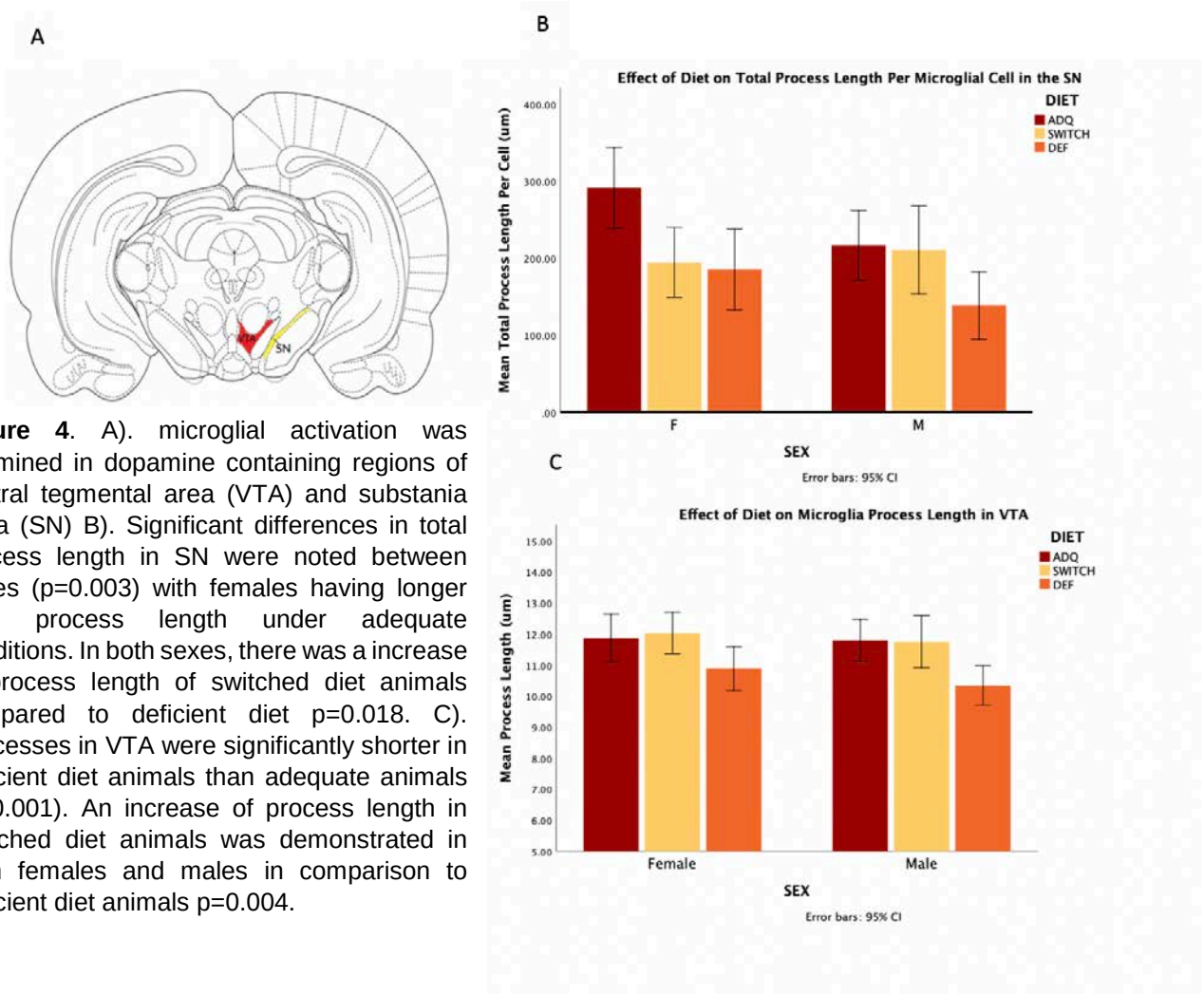

Figure 4. A). microglial activation was examined in dopamine containing regions of ventral tegmental area (VTA) and substania nigra (SN) B). Significant differences in total process length in SN were noted between sexes (p=0.003) with females having longer total process length under adequate conditions. In both sexes, there was a increase in process length of switched diet animals compared to deficient diet p=0.018. C). Processes in VTA were significantly shorter in deficient diet animals than adequate animals (p=0.001). An increase of process length in switched diet animals was demonstrated in both females and males in comparison to deficient diet animals p=0.004.

The analyzed data (presented above) so far indicates that n3 PUFA deficiency significantly affects microglial activation in some dopamine containing regions, and that some these effects may be ameliorated by supplementation. Further analysis is required as trends vary among brain regions. VTA and SN show significant positive changes in process length post supplementation, while striatal regions demonstrate no change between supplemented and deficient animal microglia morphology. Effects of diet are seen in all brain regions in both sexes. Sex differences in response to adequate and deficient diets in females exhibit a more pronounced process length variance in comparison to male animals. Data collection is continuing to assess sex differences and microglial activation in other brain regions.

What opportunities for training and professional development has the project provided?

Postdoctoral training of personnel involved in the project

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

1. Continue Major Task 2 to provide sufficient subjects complete behavioral data collection for deficient and adequate diet cohorts

2. Complete Major Task 3

3. Complete Major Task 4

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. **CHANGES/PROBLEMS:**
Changes in approach and reasons for change

None. The changes we made last year to automate behavioral data collection and quantification of microglia activation in specific brain regions were successful. We were rapidly collected data before SARS-Cov2 related laboratory shutdown. We anticipate completing the proposed work in the next 4-6 months.

Actual or anticipated problems or delays and actions or plans to resolve them

A temporary hold on breeding was placed in response to institutional shutdown due to SARS-Cov2 pandemic. Colony growth of first and second generation special diet animals is resuming and behavior testing will be conducted within the next few months.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Nothing to report

  Journal publications.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers, and presentations.

  Nothing to report
- **Website(s) or other Internet site(s)**
  Nothing to report
- **Technologies or techniques**
  Nothing to report
- **Inventions, patent applications, and/or licenses**
  Nothing to report
- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Bita Moghaddam, PhD
Project Role: Partnering PI
Nearest person month worked: 1 calendar month
Contribution to Project: Dr. Moghaddam supervised the project, including completion of protocols, overseeing all aspects of animal testing and data analysis.

Kathryn Wallin-Miller, PhD
Project Role: Postdoctoral Researcher
Nearest person month worked: 9 calendar months
Contribution to Project: Dr. Wallin-Miller has established the new method of microglia assessment and was responsible for post-processing analysis of the tissue for Major Task 3.

Nicole Kahn
Project Role: Research Assistant
Nearest person month worked: 7 calendar months
Contribution to Project: Ms. Kahn was responsible for all breeding (Task 2) and assisting with behavior testing and analysis (Tasks 3).

Alina Bogachuk
Project Role: Research Assistant
Nearest person month worked: 2 calendar months
Contribution to Project: Ms. Bogachuk is currently responsible for all breeding (Task 2) and assisting with behavior testing and analysis (Tasks 3 initial stages of tissue processing.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No changes in senior/key personnel

Changes in active support for PI Bita Moghaddam:

NEW

None

Ended (closed)

R56 MH084906 - 06A1 (Moghaddam) 7/01/17 – 6/30/19 (NCE) 2.76 calendar
NIMH/NIH $320,019
"Inhibitory Control of Prefrontal Cortex"
Anxiety is a debilitating symptom of most psychiatric disorders including PTSD, major depression, and addiction. The proposed studies aim at understanding the neuronal basis of anxiety and its impact on goal-directed behavior.
Role: PI

R01MH048404 (Moghaddam) 9/15/92 – 6/30/19 3.4 calendar
NIMH/NIH $250,000
"Neurochemical Effects of Antipsychotic Drugs"

The goal of this grant is to investigate the mechanisms by which disruptions to cortical systems affect corticolimbic circuitry.
Role: PI

OVERLAP

There is no overlap.

Other organizations involved as partners:

University of Pittsburgh
Pittsburgh, Pennsylvania
Partner's Contribution to the project:
Collaboration

This award involved a Partnering Award at the University of Pittsburgh, Partnering PI: Dr. Rajesh Narendran. Dr. Narendran will submit an independent progress report per the instructions for collaborative awards. There are no additional organizations involved as partners.

8. SPECIAL REPORTING REQUIREMENTS

Partnering PI report will be filed separately for Aim 1 (human study) by Dr. Rajesh Narendran, University of Pittsburgh, Pittsburgh, PA

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.
N/A

9. APPENDICES:

Nothing to report